

MONASCUS LUNISPORAS, A NEW SPECIES ISOLATED FROM MOULDY FEEDS

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ABSTRACT : A new species of *Monascus* (Ascomycotina; *Monascaceae*), *M. lunisporas* is described and illustrated. This species is characterized by its dark olive-brown ascomata with an areolate wall, lunate ascospores and hyaline to olive-brown, often verrucose conidia. The holotype was isolated from mouldy feeds in Tokyo. A key to *Monascus* species is provided.

KEY WORDS : Ascomycotina, feed, *Monascus lunisporas*, taxonomy.

RÉSUMÉ : Une espèce nouvelle de *Monascus* (Ascomycotina, *Monascaceae*), *M. lunisporas*, est décrite et illustrée. Elle se distingue par ses ascomata brun olivacé sombre présentant une paroi aréolée, des ascospores en forme de lune et hyalines à brun sombre et la production de conidies à paroi souvent verruqueuse. Le matériel original a été isolé de fourrages moisissés dans la ville de Tokyo. Une clé de détermination des espèces du genre est proposée.

MOTS CLEFS : Ascomycotina, fourrage, *Monascus lunisporas*, taxonomie.

INTRODUCTION

The ascomycete genus *Monascus* is well known as the organisms used in Asia to prepare pigmented red rice (ang-kak), fermented soybean curd, kaoliang brandy and rice wine, and is usually placed in the *Monascaceae*, in the Eurotiales (Eriksson & Hawksworth, 1993; Hawksworth *et al.*, 1995). Members of the genus are also known as food contaminants. As such, they are repeatedly encountered among the xerophilic fungi isolated from various cereals and cereal products, cooked potatoes, starch, spices, dried fish, dried fruits, crude sugar and honey (Domsch *et al.*, 1980; Samson *et al.*, 1996; Pitt &

Hocking, 1997). They are also dominant members of the mycoflora in maize, and various feedstuffs and silage for livestock.

During our microbiological examination of causal agents associated with feed spoilage, numerous isolates of *Monascus* were obtained from the dilution agar plates of one pellet sample of feeds for racing horses. Besides strains of *M. ruber* van Tieghem, an unusual member of *Monascus* was identified, characterized by dark-colored ascomata with an areolate wall and lunate ascospores. This species can not be assigned to any of the currently accepted taxa of the genus (Hawksworth & Pitt, 1983; Barnard & Cannon, 1987; Hocking & Pitt, 1988; Cannon *et al.*, 1995) and is, therefore, being described as new.

MATERIAL AND METHODS

The isolate was derived from a single colony developed on potato-dextrose agar plate by the dilution plating technique during the course of a microbiological examination of spoiled pellet feeds for racing horses. A representative culture has been deposited at the American Type Culture Collection, Manassas, Virginia, USA (ATCC). A dried culture to serve as holotype has been deposited at the Natural History Museum and Institute, Chiba, Japan (CBM). An isotype is on deposit at the "Collections du Laboratoire de Cryptogamie du Muséum National d'Histoire Naturelle, Paris, France (PC)".

Cultural characteristics were based on plate cultures grown on Czapek yeast extract agar (CYA), malt extract agar (MEA), Harrold's agar (M40Y) and 25% glycerol nitrate agar (G25N). Plastic petri dishes, 9 cm × 15mm, were used. Culture plates were incubated at 5°C, 25°C and 37°C in darkness. In the description, colour names and nomenclature were taken from Kornerup & Wanscher (1978) and Rayner (1970), designated as "M" and "R" respectively. The enzyme activity tests were carried out using the APIZYM system, following the methods of Bridge & Hawksworth (1985).

TAXONOMY

Monascus lunisporas Udagawa et Baba, *sp. nov.*

figs. 1-9.

Coloniae in agarō maltoso modice effusae, radiatim sulcatae, tenues, ex mycelio vegetativo submerso constantes, ascomatibus numerosis et hyphis aeriis sparsis formantes, griseo-brunneae vel olivaceo-brunneae vel griseo-olivaceae; conidiogenesis moderata vel conspicua; reversum olivaceo-brunneum vel griseo-olivaceum.

Mycelium ex hyphis hyalinis vel dilute olivaceo-brunneis, ramosis, septatis, tenuibus, levibus, 2-6(-14) μm diam, saepe fasciculatis compositum. Conidia solitaria vel breviscatenata, hyalina vel olivaceo-brunnea, unicellularia sed interdum transverse uniseptata, globosa vel subglobosa vel obovoidea, 6-12(-16) μm diam, incrassata, levia vel saepe verrucosa, basi truncata.

Ascomata superficialia, dispersa, non ostiolata, valde olivaceo-brunnea, globosa, 40-90 μm diam, saepe brevi-stipitata, celeriter maturescentia; peridium tenue, primo trans-

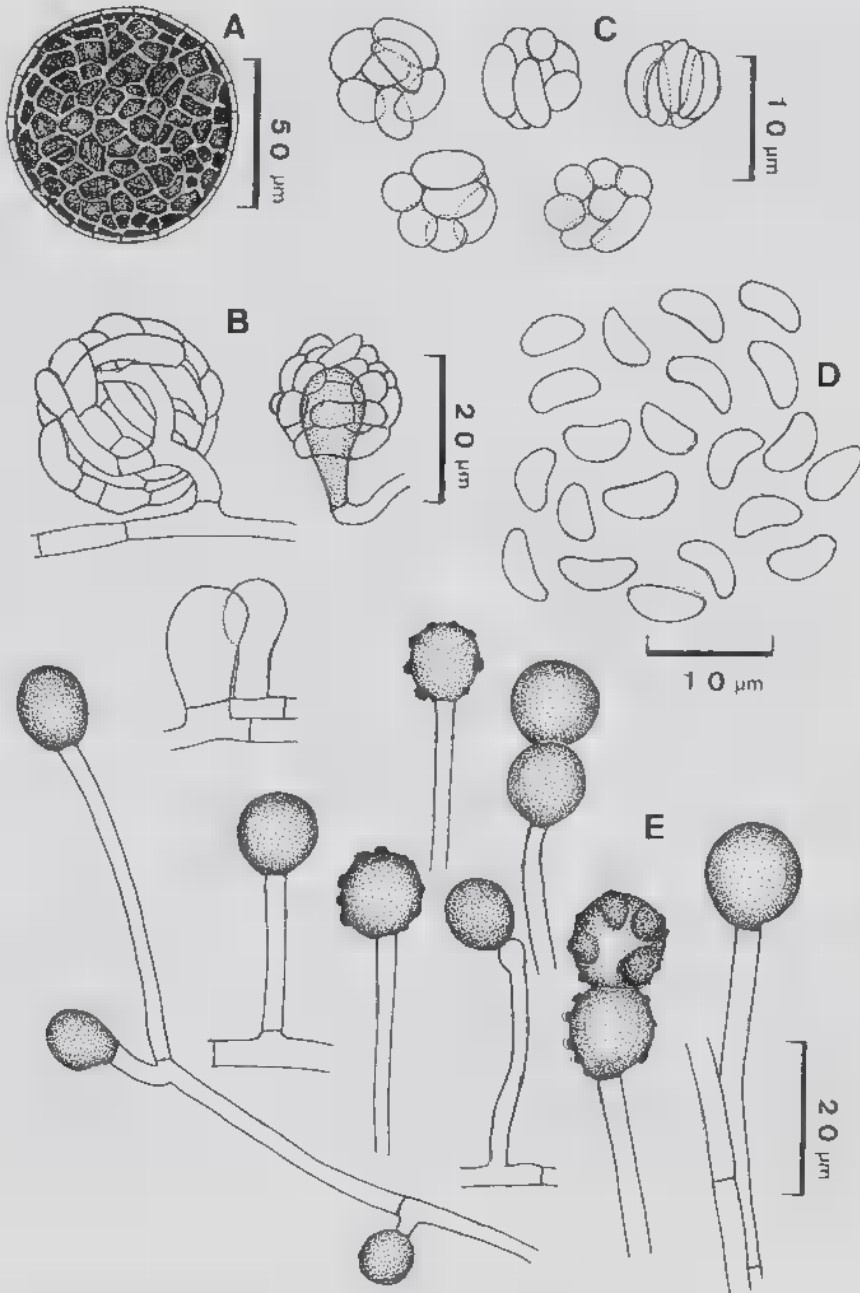
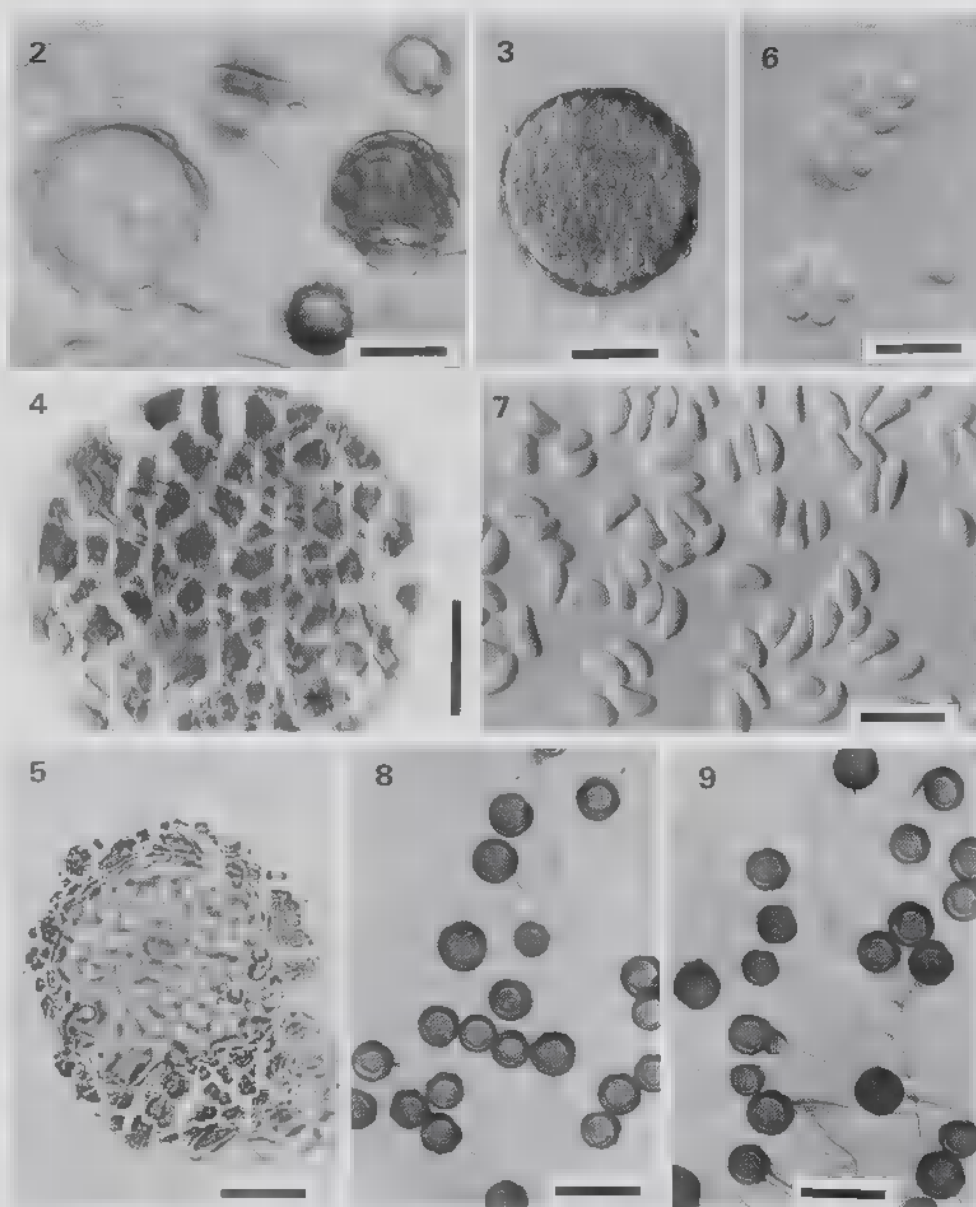


FIG. 1. *Monascus lunisporas*, SUM 3116.
 A. Mature ascoma, B. Ascomatal initials and young ascoma,
 C. Asci, D. Ascospores, E. Conidium formation at the tip of hyphae.



FIGS. 2-9. *Monascus lunisporus*, SUM 3116.

2. Young ascomata, 3. Mature ascoma, 4. Peridium showing areolate outer layer, 5. Peridium cross section, 6. Asci, 7. Ascospores, 8, 9. Conidium formation at the tip of hypha.

Bars: 2, 6, 7 = 10 μm ; 3, 4, 5, 8, 9 = 20 μm .

lucens, deinde olivaceo-brunneum vel valde olivaceo-brunneum; ex strato exteriori areolato et strato interiore membranaceo compositum. Asci octospori, globosi vel subglobosi, 9-10 (-10.5) × 8-9 μm, mature evanescentes. Ascospores hyalinae, lunatae, interdum ellipsoideae, 5-7 × 2.5-3 μm, aliquantum incrassatae, non septatae, leves.

Holotypus. SUM 3116: colonia exsiccata in cultura ex pabulo mucido. Tokyo, in Japonia, 19.iii.1998, a H. Baba isolata et ea collectione fungorum Musei et Instituti Historiae Naturalis Chiba (CBM) conservata.

Etymology: from Latin, *luni* = crescent and *spora* = seed, referring to the ascospore shape.

Colonies on CYA growing restrictedly, attaining a diameter of 7-10 mm in 7 days at 25°C, plane, thin, vegetative mycelium submerged, producing ascomata in limited numbers and sparse aerial hyphae, more or less funiculose, Greyish Brown (M 8E3) or Chestnut (R); conidiogenesis inconspicuous; reverse Greyish Brown to Dark Brown (M 8F3-4) or Sepia (R), with surrounding agar lightly colored.

Colonies on MEA growing rather rapidly, attaining a diameter of 18-23 mm in 7 days at 25°C, radially sulcate, thin, vegetative mycelium submerged, with surface developed numerous ascomata and sparse aerial hyphae, Greyish Brown to Olive Brown (M 5-4E3) or Grey Olivaceous (R); margins irregularly dissected, thin; conidiogenesis moderate to conspicuous; soluble pigments not produced; reverse Olive Brown (M 4F4) or Grey Olivaceous (R).

Colonies on M40Y rapidly growing, attaining a diameter of 25-26 mm in 7 days at 25°C, plane, floccose, consisting of a thin basal felt and loose aerial hyphae, at first white but after one week rapidly developing abundant ascomata over most of the colony surface and becoming Olive Brown to Dark Green (M 4E3-30F5) or Olivaceous Grey (R); margins more or less irregular, broad, thin; conidiogenesis prominent; reverse Dark Green (M 29F4) or Iron Grey (R).

Colonies on G25N growing slowly, attaining a diameter of 5-7 mm in 7 days at 25°C, radially sulcate but with marginal areas usually plane and thin, somewhat funiculose; ascoma formation delayed and reduced, Olive Grey (M 3E2); conidiogenesis moderate; reverse Brownish Grey (M 5F2).

At 5°C, growth is nil.

At 37°C, CYA and MEA in 7 days: colonies 6-7 mm in diameter and 11-13 mm in diameter respectively; conidial development is sparse and ascomata are not appeared.

Mycelium composed of hyaline to pale olive-brown, branched, septate, thin-walled, smooth, 2-6(-14) μm diam hyphae, often forming bundles. Conidia formed singly or in short basipetal chains by swelling of the apex of unmodified hyphae, hyaline to olive-brown, one-celled, sometimes transversely 1-septate, globose to subglobose or obovoid, 6-12(-16) μm in diam, very thick-walled, smooth to often verrucose, truncated at the base due to the formation of secession scar.

Ascomata arising singly from a central cell being a swollen lateral branch of the hypha and around which a narrower hypha tightly coils; ascomata superficial, scattered, non-ostiolate, dark olive-brown, globose, 40-90 μm in diam, often short-stipitate, maturing rapidly; peridium thin, 3-6 μm thick, formed from brown, septate, thick-walled, ramifying hyphae 2.5-7 (-10) μm wide; peridial hyphae fuse to develop olive-brown to dark olive-brown, polygonal plates, areolate, made of two distinct tissue layers in cross section; peridial cells of the outer layer dark olive-brown, thick-walled, irregularly angular in surface view, 4-10 μm in diam, forming a layer 1-2 cells deep in cross section; peridial

cells of the inner layer hyaline to pale-colored, translucent, thin, membranous. Asci 8-spored, globose to subglobose, $9-10(-10.5) \times 8-9 \mu\text{m}$, early evanescent. Ascospores hyaline, lunate, sometimes ellipsoidal, $6-7 \times 2.5-3 \mu\text{m}$, rather thick-walled, aseptate, smooth.

Holotype. SUM 3116: dried culture derived from an isolate from mouldy feeds for racing horse, Tokyo, Japan, 19 March 1998, collected by H. Baba. The type specimen is deposited in the Natural History Museum and Institute, Chiba, Japan (CBM).

Note. The outstanding features of *M. lunisporas* are: (1) distinctly rapid growth on MEA at 25°C, (2) dark coloration on MEA-Greyish Brown to Olive Brown ascomata and conidial masses, and lack of soluble pigments, (3) large olive-brown ascomata with an areolate wall, (4) hyaline lunate ascospores, and (5) often olive-brown and verrucose, thick-walled conidia.

Since the most recent revision of the genus by Hawksworth & Pitt (1983), 7 species have been described and accepted: *M. eremophilus* Hocking & Pitt, *M. floridanus* P.F. Cannon & Barnard, *M. pallens* P.F. Cannon, Abdullah & B.A. Abbas, *M. pilosus* K. Saito ex D. Hawksw-S. Pitt, *M. purpureus* Went, *M. ruber* and *M. sanguineus* P.F. Cannon, Abdullah & B.A. Abbas (Barnard & Cannon, 1987; Hocking & Pitt, 1988; Cannon *et al.*, 1995).

The new species apparently differs from the former in ascospore characteristics as well as in areolate structure of the ascomatal wall and in conidial ornamentation. The enzymic activity of the new species is also similar to those of *M. floridanus* and *M. sanguineus*, but it can be distinguished from the first by lack of trypsin activity, and from the second by weakly positive leucine of arylamidase and absence of valine arylamidase.

The ascospores of *M. lunisporas* are reminiscent of those of *Xeromyces* L. R. Fraser in being crescent shape, which is another genus in the *Monascaceae* and has a single species, *X. bisporus* L.R. Fraser (Fraser, 1953; Udagawa *et al.*, 1986; Pitt and Hocking, 1997). *Xeromyces bisporus* is unique in its lowest requirement for available water (0.61 a_w) of any known organism (Pitt and Hocking, 1997). Arx (1974) considered *Monascus* and *Xeromyces* as indistinguishable, and *X. bisporus* was transferred to *Monascus*. However, *X. bisporus* is distinctive in having almost colorless, sessile ascomata, different ascomatal initials which start as a short lateral branch of the hypha, become septate and then develop into a central cell and four holding branches, 2-spored asci, and a *Fraseriella* anamorph (Fraser, 1953; Corte, 1957).

KEY TO SPECIES OF *MONASCUS*

1. Ascospores lunate, $5-7 \times 2.5-3 \mu\text{m}$; ascomata dark olive-brown *M. lunisporas*
1. Ascospores ellipsoidal or broadly ellipsoidal; ascomata hyaline or pigmented 2
 2. Ascospores small, less than $5 \mu\text{m}$ long 3
 2. Ascospores large, exceeding $5 \mu\text{m}$ long 4
 3. Ascomata and conidia remaining hyaline; ascospores $3.5-4 \times 2.5-3 \mu\text{m}$ *M. pallens*
 3. Ascomata and conidia brown to dark brown; ascospores $3.5-4.5 \times (2-) 2.5-3 \mu\text{m}$ *M. floridanus*

4. Obligately xerophilic (no growth on CYA and MEA); ascomata brownish orange; ascospores $(5-)-6-7 \times (5-)-5.5-6.5 \mu\text{m}$ *M. eremophilus*
4. Not obligately xerophilic (colonies on MEA at least attaining a diameter of 8 mm at 25°C in 7 days) 5
5. Ascomata and conidia remaining hyaline 6
5. Ascomata and conidia pigmented 7
6. Colonies on MEA exceeding 28 mm diameter at 25°C in 7 days; ascospores ellipsoidal, $5-7(-8.5) \times 3-3.5(-4) \mu\text{m}$ *M. pilosus*
6. Colonies on MEA not exceeding 24 mm diameter at 25°C in 7 days; ascospores broadly ellipsoidal, $(5.5-)-6-7 \times (4-)-4.5-5 \mu\text{m}$ *M. purpureus*
7. Colonies on MEA exceeding 28 mm diameter at 25°C in 7 days; ascomata brownish orange but soluble pigments not produced; ascospores $5-6.5(-7.5) \times (3.5-)-4-4.5 \mu\text{m}$ *M. ruber*
7. Colonies on MEA not exceeding 12 mm diameter at 25°C in 7 days; blood-red soluble pigments produced; ascospores $6-7(-7.5) \times 4-4.5 (-5) \mu\text{m}$ *M. sanguineus*

ACKNOWLEDGEMENTS: We wish to express our gratitude to Dr. J. Mouchacca for providing some information and to Mr. S. Uchiyama for technical assistance.

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